REMARKS

Claims 1-26 are all the claims pending in the application. Claims 9-26 are non-elected claims and are withdrawn from consideration. Claims 1-8 are all the claims under examination.

The amendment to claim 1 further clarifies the scope of the present invention and specifically defines the functional activity of the claimed protox enzyme. Support for this amendment may be found at pages 24-25, Example 7 and Table 6, of the present specification. The amendment to claims 1-8 distinguishes the claimed invention from a product of nature. Support for this amendment may be found throughout the specification, in particular at Example 3, pages 16-21. Further amendments to claims 1-8 correct grammatical errors, correct antecedent and formatting errors, and place the claims in proper dependent form.

Applicants respectfully submit that the amendments are fully supported and no new matter has been introduced, hence Applicants respectfully request entry of the same.

Consideration of references

At page 2 of the Office Action, the Examiner stated that five (5) references cited in Applicants' Information Disclosure Statement of March 13, 2000 were not present in the application and therefore were not considered by the Examiner.

Applicants note that the five (5) references in question were cited in the International Search Report of the PCT application (PCT/JP/04064). When a national stage application is filed under 35 U.S.C. § 371, Applicants are not required to submit a copy of any reference cited in an international search report. Documents cited in an international search report are generally sent by the Searching Office to the USPTO and are made available to the examiner in the

national stage application. Further, Applicants received verification that a copy of the International Search Report and copies of the references cited therein was received by the USPTO (i.e., Notification of Acceptance of Application under 35 U.S.C. § 371 (Form PCT/DO/EO/903) dated June 15, 2001, a copy of which is enclosed herewith). Applicants therefore respectfully ask that the Examiner review the five references received by the USPTO in its capacity as a Designated Office under 37 C.F.R. § 1.494, and acknowledge the consideration of each on the copy of the Form PTO-1449 also included herewith.

Drawings

At page 2, paragraph 1, of the Office Action, the Examiner notes that the drawings submitted with this application were objected to by the Draftsperson. In response, Applicants submit corrected drawings herewith and respectfully request acknowledgement of the same.

Title of the invention

At page 2, paragraph 2, the Examiner objects to the title of the invention and suggests an alternative. Applicants have amended the title of the invention as suggested by the Examiner, and thereby request reconsideration and withdrawal of this objection.

Claim Objections

A. At page 3, paragraph 3, the Examiner objects to claims 6-8 under 37 C.F.R. § 1.75(c). Applicants have canceled claim 6 and amended claims 7-8 to place them in proper dependant form, and thereby request reconsideration and withdrawal of this objection.

B. At page 3, paragraph 4, the Examiner objects to claims 3 and 4 as using poor grammar. Applicants have amended claims 3 and 4 to correct this error, and thereby request reconsideration and withdrawal of this objection.

C. At page 3, paragraph 5, the Examiner objects to the improper format of the term "SEQ ID No.2" in claims 1-5. In the amendment to claims 1-5, Applicants have replaced the incorrect term with "SEQ ID NO: 2", and thereby request reconsideration and withdrawal of this objection.

Claim Rejection under 35 U.S.C. § 101

At page 3, paragraph 6, the Examiner rejects claims 1-8 under 35 U.S.C. § 101. The Examiner suggests amending the claims to indicate activity by the "hand of the inventor". Applicants have canceled claim 6 and amended claims 1-5 and 7-8 to recite an "isolated" protoporphyrinogen oxidase. Thus, Applicants request reconsideration and withdrawal of this rejection.

Claim rejection under 35 U.S.C. § 112, second paragraph

At page 4, paragraphs 7-10, of the Office Action, the Examiner rejects claims 1-8 under 35 U.S.C. § 112, second paragraph.

A. Specifically, at paragraph 8, the Examiner states that the term "substantially" should be deleted. Applicants have amended claims 1-4, and deleted the term "substantially", as suggested by the Examiner, and request reconsideration and withdrawal of this aspect of the rejection.

B. At paragraph 9, the Examiner states that in claims 2-5, there is improper antecedent basis for the limitation "the protophyrinogen oxidase". In response, Applicants have amended claims 2-5 to depend from claim 1. These claims now read in part, "The protophyrinogen oxidase ... of claim 1". Thus, claim 1 provides the proper antecedent basis for these dependent claims. Based upon this amendment, Applicants request reconsideration and withdrawal of this aspect of the rejection.

C. At paragraph 10, the Examiner rejects claims 3 and 4, as being unclear as to what amino acids compose the "transit peptide" recited in these claims. In response, Applicants note that "transit peptide" is described at page 3 of the specification. A transit peptide is a type of "signal sequence" that targets proteins to different destinations (i.e., organelles and compartments) within the cell. It is a short region of the protein defined by general physical characteristics, such as charge and hydrophobicity, but in which the amino-acid sequence varies within these constraints. The transit peptide sequence is cleaved from the protein after it has served its targeting purpose. As Applicants describe in the specification, the transit peptide is usually encoded at the N-terminal end of the protein and upon cleavage, the principle biological activity remains with the mature protein.

One of ordinary skill in the art could readily determine which amino acids of SEQ ID NO:2 comprise the transit peptide. Thus, the delineation of "transit peptide" in claims 3 and 4 is clear enough so that it does not require the additional identification of specific amino acids. In view of these comments, Applicants respectfully request reconsideration and withdrawal of this aspect of the rejection.

Claim rejection under 35 U.S.C. § 112, first paragraph

A. At page 4, paragraph 11, of the Office Action, the Examiner rejects claims 1, 3 and 6-8 under 35 U.S.C. § 112, first paragraph.

To more clearly define their invention, Applicants have amended claim 1 to encompass protox polypeptides of equivalent "tolerance to pyrazole compounds". In addition, Applicants have amended claims 1-4 to delete the term "substantially". Applicants further amend claim 3 to depend from claim 1, cancel claim 6, and amend the corresponding dependencies of claims 7 and 8.

In view of the foregoing reasons, and Applicant's amendments to claims 1-4 further defining the claimed invention, the Examiner's rejection should be withdrawn. Applicants respectfully request reconsideration and withdrawal of this rejection.

B. At page 5, paragraph 12, the Examiner rejects claims 1, 3, and 6-8 under 35 U.S.C. § 112, first paragraph.

The Examiner alleges that Applicants claim any protox peptide tolerant to any photobleaching herbicide. In contrast, Applicants assert that the present application essentially claims the protox polypeptide of SEQ ID NO:2 and peptides with equivalent "tolerance to pyrazole" and equivalent "enzyme activity". Applicants describe equivalent enzyme activity as protox activity at an equivalent level to that of SEQ ID NO:2 peptide, across a spectrum of compounds and concentrations. Applicants recite specific functional elements that limit the scope of the claims to protox peptides with the equivalent measured characteristics of protox activity in the absence and presence of specific herbicide compounds.

In support of an enabling specification, in Example 2 Applicants disclose cross-resistance of selected cell lines against various herbicides. The results displayed in Table 3, page 14, allowed Applicants to conclude that ETR-056 overproduced superoxide dismutase, ETR-253 had decreased membrane permeability, and ETR-245 had a mutated protox gene. Example 3-C, at page 17, provides a predictable means for one skilled in the art to screen for mutant peptides resistant to herbicide compounds. Such experimentation would not require undue experimentation. Thus, Applicants disclose a rational scheme for modifying protox peptides, so that one of ordinary skill in the art could practice the claimed invention.

In view of Applicants' amendments to claim 1-4, and the foregoing comments, the Examiner's rejection should be withdrawn. Applicants respectfully request reconsideration and withdrawal of this rejection.

Claim rejection under 35 U.S.C. § 102

A. At page 7 of the Office Action, paragraph 13, the Examiner rejects claims 1, 3, and 6-8 under 35 U.S.C. § 102(b). The cited reference is Ward et al. (WO95/34659).

In response, Applicants assert that the claimed protox peptide is distinguishable from that described in Ward et al. Ward et al. describe a protox enzyme from *Arabidopsis thaliana* and *Z. may*, and do not disclose a protox enzyme from *Nicotiana tabacum*, as disclosed by Applicants. Further, the resistance mutation described in Ward et al. achieved only 10X increased resistance compared to the wild type strain. The resistance mutation of Applicants' present invention achieved much more than 10X resistance, in fact it has as much as several thousand fold increased resistance (Table 6). Applicants' own experimental evidence demonstrates that their

mutant tobacco protox enzyme has a vast superiority over mutant *A. thaliana* (Table 6). Thus, the disclosure of Ward et al. does not anticipate the present invention.

Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

B. At page 8, paragraph 14, the Examiner rejects claims 1, 3 and 6 under 35 U.S.C. § 102(e). The cited reference is Volrath et al. (US 5,939,602).

Applicants assert that Volrath et al. describe protox polypeptides from a variety of sources and an assortment of "modified" forms of protox polypeptide. Although Volrath et al. characterize these modified polypeptides as, for example, "tolerant to a herbicide in amounts that inhibit the naturally occurring protox activity" Volrath et al. fail to directly measure protox enzyme activity. Volrath et al. identify proposed mutated forms of protox only by screening for bacterial cells that tolerate higher concentrations of herbicide (Examples 9-18). Volrath et al. then use some of these native and mutated forms of protox to produce herbicide tolerant plants (Examples 29-33).

In order for the Examiner to make a rejection using a reference under 35 U.S.C. § 102(e), every element of the rejected claims must be contained in the reference. The Volrath et al. reference fails to anticipate the instant invention because it does not disclose a protox having an enzyme activity *substantially equivalent* to a polypeptide represented by SEQ ID NO:2. Applicants use the term "an enzyme activity" in claims 1-4 to mean protox activity, specifically meaning that any altered form of SEQ ID NO:2 protein must retain the substantially equivalent

protoporphyrinogen oxidase enzyme activity. Applicants provide experimental evidence directly measuring protox enzyme activity (Table 4). Applicants further disclose data using a variety of herbicide compounds and concentrations (Table 6). Although Volrath et al. describe the screening of transformed bacterial colonies and selecting for growth on agar in the presence of herbicide, there is nothing in Volrath et al. to suggest that any of the peptides in Volrath et al. is *equivalent* to the protox peptide of SEQ ID NO:2. Thus, the Examiner has not established a prima facie case of anticipation and the Examiner's rejection should be withdrawn.

Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

Claim rejection under 35 U.S.C. § 103

At page 8 of the Office Action, paragraph 15, the Examiner rejects claims 1-8 under 35 U.S.C. § 103(a), over Ward et al. in view of Lermontova et al. (PNAS).

In response, Applicants assert that although Lermontova et al. may describe a protox enzyme of 99% homology to the polypeptide of SEQ ID NO:2, one of ordinary skill in the art would not expect that Applicants' alanine/valine substitution at position 231 would produce herbicide-resistance increased by several thousand fold (as seen in Table 6). As the Examiner is aware, Ward et al. teach that an identical amino acid substitution in two other plant species increased herbicide resistance a maximum of only 10 fold. It would not have been obvious to one of ordinary skill that the same conservative substitution in a highly homologous protein could produce the extremely high superiority of the present invention. Thus, based on the

unexpected results as shown in the present invention, the Examiner's Section 103 rejection should be withdrawn.

Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

Conclusion

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

Applicant hereby petitions for any extension of time which may be required to maintain the pendency of this case, and any required fee, except for the Issue Fee, for such extension is to be charged to Deposit Account No. 19-4880.

Respectfully submitted,

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APPENDIX

VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE TITLE:

The title is changed as follows:

Herbicide-Resistant Protoporphyrinogen Oxidase Isolated From Nicotiana tabacum.

~~Novel Protoporphyrinogen Oxidase Tolerant to Photobleaching Herbicide.~~

IN THE CLAIMS:

Claim 6 is canceled.

The claims are amended as follows:

1. (Amended) An isolated protoporphyrinogen oxidase tolerant to photobleaching herbicide and derivatives thereof, comprising a polypeptide having the amino acid sequence represented by SEQ ID NO: 2 ~~No.2~~ or a mutated peptide having deletion, addition, substitution, etc. of one or more amino acids in the above amino acid sequence and having an enzyme activity and tolerance to pyrazole compounds ~~substantially~~ equivalent to that of said ~~purified~~ protoporphyrinogen oxidase tolerant to photobleaching herbicide.

2. (Amended) The isolated protoporphyrinogen oxidase tolerant to photobleaching herbicide and derivatives thereof of claim 1, comprising a polypeptide having the amino acid sequence represented by SEQ ID NO: 2 ~~No.2~~, wherein one or more amino acids is deleted and

the polypeptide has an enzyme activity ~~substantially~~ equivalent to that of said ~~purified~~ protoporphyrinogen oxidase tolerant to photobleaching herbicide.

3. (Amended) The isolated protoporphyrinogen oxidase tolerant to photobleaching herbicide and derivatives thereof of claim 1, comprising a polypeptide having the amino acid sequence represented by SEQ ID NO: 2 ~~No.2~~, from which a transit peptide is deleted and one or more amino acids is deleted, added or substituted, and the polypeptide has ~~have~~ an enzyme activity ~~substantially~~ equivalent to that of said ~~purified~~ protoporphyrinogen oxidase tolerant to photobleaching herbicide.

4. (Amended) The isolated protoporphyrinogen oxidase tolerant to photobleaching herbicide and derivatives thereof of claim 1, comprising a polypeptide having the ~~an~~ amino acid sequence represented by SEQ ID NO: 2 ~~No.2~~, from which a transit peptide is deleted, and the polypeptide has ~~have~~ an enzyme activity ~~substantially~~ equivalent to that of said purified protoporphyrinogen oxidase tolerant to photobleaching herbicide.

5. (Amended) The isolated protoporphyrinogen oxidase of claim 1, comprising an amino acid sequence represented by SEQ ID NO:2 ~~No.2~~.

7. (Amended) The isolated protoporphyrinogen oxidase according to any one of claims 1-5, wherein the photobleaching herbicide is a compound selected from the group consisting of

ethyl 2-chloro-5-(4-chloro-5-difluoromethoxy-1-methyl-1H-pyrazole-3-yl)-4-fluorophenoxyacetate, ethyl 2-[5-(4-chloro-5-difluoromethoxy-1-methyl-1H-pyrazole-3-yl) -2,4-dichloro-phenylamino]propionate, 4-chloro-3- [4-chloro-2-fluoro-5-methoxyphenyl)-5-difluoromethoxy-1-methyl-1H-pyrazole, 4-chloro-3- [4-chloro-2-fluoro-5- (2-propynyl) oxyphenyl] -5-difluoromethoxy-1-methyl-lH pyrazole, ethyl 2- [2-chloro-5- (4-chloro-s-difluoromethoxyl methyl-1H-pyrazole-3-yl) -4-fluorophenoxy]propionate and l-methylethyl 5-[4-bromo-1-methyl-5- (trifluoromethyl)-1H-pyrazole-3-yl]-2-chloro-4-fluoro-benzoate, 4-chloro-3-(4-chloro-2-fluorophenyl)-5-difluoromethoxy-1-methyl-1H-pyrazole.

8. (Amended) The isolated protoporphyrinogen oxidase according to claim 7 ~~Claims 1-5~~, wherein the photobleaching herbicide is ethyl 2-chloro-5- (4-chloro-5-difluoromethoxy-1-methyl-1H-pyrazole-3-yl)-4-fluoophenoxyacetate.